

DOMESTICATING GROUND HEMLOCK (*Taxus canadensis*) FOR PRODUCING TAXANES: A CASE STUDY

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ABSTRACT

The secondary plant product, paclitaxel (Taxol[®]), is a diterpenoid isolated from the foliage and roots of a number of different yew species for the treatment of cancer. Ground hemlock (*Taxus canadensis*) is one of the last unexploited yew species in the world, but with the increase in the demand for paclitaxel and related compounds, the potential threat exists that *T. canadensis* may be over-harvested, as has happened elsewhere. Since 1997, the Canadian Forest Service – Atlantic Forestry Centre has engaged in a program to minimize over-utilization of ground hemlock by research in (1) developing ecologically sustainable harvesting protocols in natural stands, and (2) by a domestication program to convert elite cultivars of the wild species into a commercially reared crop.

To ensure the sustainability of the woodland harvest, yet accommodate the industrial need for reasonable biomass collection practices, a ground hemlock working group was formed with government, industry and environmental NGO representatives. The working group has devised and agreed to harvest based on a set of minimum acceptable sustainable harvest standards. In a second group initiative, a series of collaborative research plots are being installed in 2002/2003 to assess different harvest practices.

A CFS-AFC domestication project using tree improvement methodology is being used to convert ground hemlock into a nursery crop involving a) selecting individuals from throughout the species range, b) testing and screening to select varieties with a high taxane content, and then c) implementing a large-scale production program for these "elite" plants. Research is ongoing in five interdependent yet complementary areas: a) cultivar selection, b) nursery propagation with rooted cuttings, c) intensive nursery culture, d) tissue culture, and e) methods to culturally increase taxane yields.

An integrated multi-disciplinary approach is being developed to produce low-cost, high-quality biomass that can compete globally with countries elsewhere where labor costs are exceedingly low compared with North America. Until significant quantities of domesticated biomass can be harvested, the woodland resource is being preserved by implementing sustainable harvesting methods. It is suggested that the *T. canadensis* ground hemlock domestication program can be used as a model for bioproducts from other woody species found in the region.

Introduction

Since the 1960s, the U.S. National Cancer Institute (NCI) has maintained a research and development program in which plants from all over the world, including hundreds of native North American forest plants, are sampled in an effort to find natural compounds for use in the fight against cancer. One of the promising substances the NCI has found was paclitaxel (Taxol[®]). Initially found in the bark of the Pacific yew (*Taxus brevifolia*) tree, this "chemical gem" has been shown to be very effective against breast, ovarian, and non-small cell lung cancers. Over the past 10+ years, paclitaxel and some of the other 'taxanes' have shown promise for use against more than 20 cancer and non-cancer diseases. According to the U.S. National Institute of Health, paclitaxel is the most important anti-cancer drug to have been marketed in the last 15 years.

Taxanes are found in all yew species throughout the world, but the specific compounds and their concentrations vary greatly among species. The relatively high value of taxane-yielding biomass has resulted in significant reductions in native populations of virtually all of the other yew species throughout the world. The tissues (roots, bark, and foliage) of ground hemlock or eastern yew (*Taxus canadensis*)

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contain adequate concentrations of paclitaxel but, more importantly, they contain abundant quantities of several related taxane compounds that may either be chemically converted into paclitaxel, or serve as precursors for the second generation of even more potent anti-cancer medications currently being developed. As a consequence of the combination of the slow growth of this species in the forest, browsing by deer and moose, forest timber harvesting and land conversion, and a general worldwide shortage of taxane-yielding biomass, ground hemlock may be threatened with over-harvesting in the very near future. Although the demand for biomass from ground hemlock is only recent, there is reason to think that a similar pattern of resource depletion will likely occur unless pre-emptive conservation measures are put in place.

As with any other naturally occurring crop, over-utilization of ground hemlock can be minimized in two ways: (1) by developing biologically sound protocols to ensure that harvesting from natural stands is ecologically sustainable, and (2) by maximizing product yield through a domestication program. Here the term 'domestication' refers to the conversion of a wild species to a commercially reared crop (also called 'tree improvement' in forestry). In 1997, the Canadian Forest Service - Atlantic Forestry Centre initiated a research program on ground hemlock that focuses on both of these elements. This paper will provide a brief discussion of some of the continuing challenges associated with this program. A more detailed discussion regarding the issues surrounding the start-up of the domestication program can be found in these proceedings (Cameron and Smith). Most of the research projects have only recently started, so final results are not yet available. Nonetheless, some selected examples of findings are provided.

Cooperation: the Key to Protecting the Resource

Determining a sustainable harvest system that will protect the resource without "killing" a fledgling ground hemlock industry has proven to be more than a little problematic. To date, little research has been done to quantify the impact of different levels of harvesting on either the relative abundance or regrowth of ground hemlock. Considerable anecdotal information based on examining harvested plants exists, but there are only three harvesting research trials of note that have been established in eastern Canada. Although considerable effort has gone into establishing these trials, they have provided only limited information that can be used to develop harvest guidelines. Our intent is not to be overly critical, but it is important to acknowledge the limitations of the work done to date in order to adequately determine what still needs to be done.

These research trials notwithstanding, industry certainly does not as yet have results from a set of harvest research plots on which to base long-term sustainable harvest practices. Since there is more than one correct way to harvest sustainably, a number of different "sustainable harvest practices" have been adopted by those working in the sector. This lack of consistency in harvest practices has resulted in some uncertainty within governmental agencies as to what set of harvest criteria should be adopted. The lack of consistency has also hampered efforts to attract significant private sector investment, e.g., investment in value-added facilities.

In an effort to reduce this ambiguity, the Eastern Canadian Ground Hemlock Working Group (ECGHWG) was formed. The ECGHWG has representatives from the private sector, five provincial Departments of Natural Resources, academia, the federal government, and thus far, one environmental non-governmental agency (ENGO). ECGHWG members, working co-operatively, hope to increase the credibility of the fledgling biomass harvesting industry by demonstrating that harvesting is being done sustainably. In support of this goal, the group's first activity was to agree on a set of harvest guidelines titled "the Minimum Acceptable Standards for the Sustainable Harvest of Ground Hemlock". This document, while acknowledging that there is more than one way to harvest sustainably, puts sustainability of the resource at the forefront while still accommodating the industrial need for reasonable biomass collection practices.

The second and most recent activity associated with the ECGHWG is, through the CFS-AFC, establishment of a series of harvest research plots that have been designed and will be installed in 2002/2003. Harvest plots are being established at five locations: two in Quebec, and one each in Prince Edward Island, New Brunswick and Nova Scotia. Designed to complement, and not duplicate, research that has already been done, the results from these studies should, over the next 3–5 years, provide some of the answers needed to ensure sustainable harvesting over the long term.

Domestication: Expanding the Resource

A tree improvement model (forestry jargon for domestication) has been adopted for ground hemlock. This involves a) selecting a number of populations and individuals within populations, b) testing and screening these individuals in order to select high-yielding varieties, and then c) implementing a large scale production program for these "elite" plants. Since cultivated crops are typically more expensive sources of biomass than simply harvesting wild foliage, plants with higher taxane concentrations are needed if commercial cultivation is to be economically attractive. Our research project on domestication is designed to improve taxane yield through research in five interdependent yet complementary areas: a) cultivar selection, b) nursery propagation with rooted cuttings, c) intensive nursery culture, d) tissue culture, and e) methods to culturally increase taxane yields.

- a) **Cultivar selection.** Over 1325 genotypes of *T. canadensis* from across most of its natural range have been sampled and cuttings rooted. Range-wide nursery provenance trials are being established at four locations. To date, approximately 1000 clones have been planted in the tests, with the remaining scheduled to be planted in the summer of 2003. In addition to evaluating all these clones with respect to growth, they will also be screened for taxane production. Promising plant material will be selected and propagated (initially by rooted cuttings).

We have found significant variation in all growth traits, both within and among populations (Figure 1). Although based on preliminary data from nursery and greenhouse trials, estimates of the levels of within- and among-population genetic variation obtained thus far are comparable. Depending on the trait evaluated, initial estimates of within- and among-population variation levels range from 10–95 and 25–85 % respectively. Although these figures represent very preliminary estimates, they do clearly indicate that genetic gains can be made by selecting both good populations and high-performing individuals within those populations.

Total biomass production and allocation of that biomass among the plant parts (shoots and roots) has shown significant variation among genotypes. In one study with 35 clones, there was a ninefold difference in shoot biomass between the fastest and the slowest growing clones and a tenfold difference in total biomass (four plants per clone at age 4 years). Similarly, the shoot fraction (shoot dry weight/total plant dry weight) also exhibited high variation, ranging from 1.7 to 4.0.

- b) **Nursery propagation using rooted cuttings.** If a domestication program is to be successful, the time between when elite plant materials are identified (from the genetic tests) and when large-scale production of these individuals can be achieved must be minimized. Until such time that tissue culture is sufficiently developed, large-scale rooted cuttings programs will continue to be required. We have conducted a series of rooted cuttings trials that have examined the effects of:
- i) the time of year on rooting success
 - ii) the size of the cutting on rooting
 - iii) rooting hormone on speed and percentage of rooting

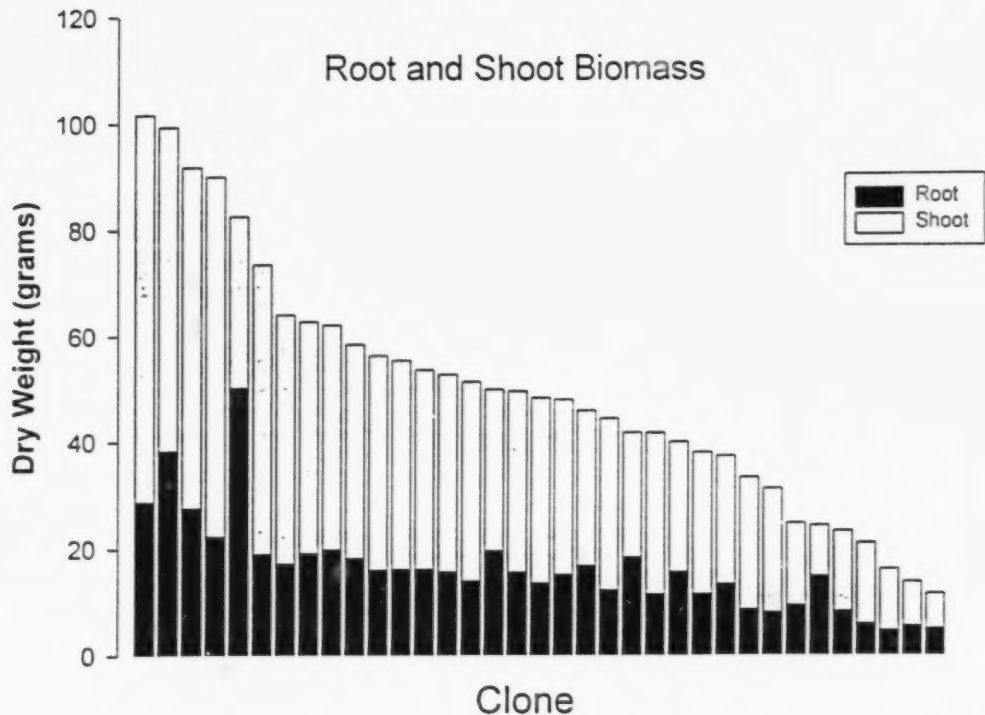


Figure 1. Total root and shoot biomass of 4-year-old ground hemlock cuttings from 35 clones. These plants were rooted and grown in pots in the nursery. Means represent two to four cuttings per clone.

We have shown that it is possible to root ground hemlock cuttings throughout the growing season, but the cultural prescriptions vary with the time of year the cuttings were struck. Although the size of the cutting had little effect on rooting percentage, the subsequent growth of the rooted plants, as might be expected, increased with increased initial size of the cutting. Throughout the rooting studies, applying rooting hormone (Seradex #3) had little if any effect on rooting success. The variation observed among genotypes masked potential effects of the rooting hormone. However, we have not conducted a thorough study using ground hemlock to compare the rooting success with and without hormone within selected genotypes. In working with other yew species under the same greenhouse conditions as for ground hemlock, we have observed the trend that the time to initiate callous is reduced slightly when rooting compound is used.

- c) **Intensive nursery culture.** One of the objectives of our research is to determine how *T. canadensis* responds to intensive nursery culture. A series of fertilizer trials are being established. Results from these studies, combined with those focusing on optimizing growing regimes, will be used to develop and support operational-scale nursery production systems. Under intensive nursery culture, we were the first group to clearly demonstrate that ground hemlock has the capacity for free growth, atypical of most "mature" northern conifers, and that clones varied considerably in this trait.

Another study underway is one in which plants are subjected to extended nursery growing seasons and intensive fertilizer, water, and top-pruning treatments. The objective of this work is to develop a system for producing potted "hedge" plants that will serve as donors for a large-scale rooted cuttings program. Over the next 3 years, we will be examining the effects, if any, of intensive nursery management on the rootability of cuttings from these plants.

- d) **Tissue culture.** An important objective of the tissue culture research program is to generate elite clonal seedlings more quickly and in greater numbers than is possible through a conventional rooted cuttings program. Research in this area includes developing methods for somatic embryogenesis (SE) and developing a bioreactor/mini-container system to produce SE tissues and plants in bulk. Work to develop a method to propagate ground hemlock using bud and root tissues is also underway. Although results from this component of the program are not yet available, early indications are promising that a viable operational-scale SE program should be available within the next 3–5 years.
- e) **Methods to increase taxane yields.** For the purposes of this program, productivity is not limited to assessing the total amount of dry weight matter per unit area per unit time. An important objective of the domestication program for ground hemlock is to maximize the amounts of taxanes produced. Therefore, plants that grow twice as fast but only produce half the taxanes, are in fact likely of lower value than plants growing slightly slower but having a modestly higher taxane content. Fast-growing, low-taxane-content plants cost more to process because more biomass must be used to produce a given amount of taxanes.

We are assessing all of the clones for their inherent capacity to synthesize taxanes. As well, quantifying taxane levels within the plants is an integral part of all the growth and development studies we are conducting. Although we have only just recently completed setting up our analytical laboratory, early results indicate levels of variation in taxane production of a magnitude comparable to what we are seeing in other traits.

In addition to screening clones for their inherent capacity to produce taxanes, we are establishing trials to determine the degree to which we can increase the taxane content of crop plants (or harvested plant tissues) through both nursery culture manipulation and pre- and post-harvest elicitation. Research trials include quantifying seasonal variation in taxane content, and relative differences among age classes of needles, and screening various materials and cultural techniques for their capacity to increase taxane production within the plant. As well, we are conducting trials directed at optimizing post-harvest handling and processing of biomass.

Ground Hemlock as a Model for Non-Timber Forest Products

In addition to ensuring that native populations of ground hemlock are not seriously depleted, successful implementation of this project can result in considerable social and economic benefits both locally and nationally. The high crop value ensures that technology developed in the project can be utilized by small-scale nurseries and niche contract tissue culture laboratories, as well as by large producers. A number of local communities may benefit through increased employment. Notwithstanding the advantage of a sustainable supply of high-yielding raw material to the Canadian and North American biopharmaceutical industry, the benefits to the public-at-large by helping to reduce the current shortage and, possibly, the high cost of paclitaxel cannot be overstated. Toward this goal, we are also collaborating with the University of New Brunswick's Faculty of Forestry and Environmental Management and Falls Brook Centre in a project to evaluate the potential of ground hemlock and other non-timber forest products in sustaining rural communities. Public education is also an integral component of this program.

Summary

The key to being successful in the plant bioactive product arena lies in employing state-of-the-art technology to increase production efficiency. An integrated multi-discipline approach (e.g., biology, genetics, chemistry and engineering) is mandatory if we are to produce a low-cost, high-quality product that can compete globally with such countries as China and India, where labor costs are exceedingly low compared with those in Canada and the United States. CFS-AFC has collaborated with a private partner in an economic analysis to demonstrate that this can be done. However, we must be seen to have a sustainably managed woodland resource now. It will take a minimum of 5 years before a large-scale "domesticated crop" can be established. In the interim, the *only* source will be from wild harvesting.

With proper marketing, we can develop a reputation in eastern Canada as a reliable source of many bioactive compounds. However, we need one solid example to demonstrate that we have the expertise to do so. The increasing demand for, and high value of, Taxol[®] means that ground hemlock is an ideal model species to begin with. We anticipate that the methods and strategies developed during the course of the ground hemlock domestication program will be transferable to other woody species with known bioproducts found in the region.

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